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Thin-Layer (Planar) Chromatography

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Introduction

Modern agricultural production in major agriculture countries depends heavily on the use of pesticides. Herbicides represent more than 50% of all pesticides used (in the USA and Germany the figure is about 60%) and are found in soil, ground and surface water and food. Triazines are the most common herbicides, and they represent *c*. 30% of all herbicides used. As they are relatively stable they are also the ones most commonly found in the environment. Triazine toxicity is low and they do not usually present a risk to humans. However, as they are extensively used, their presence in the environment and in food must be monitored (triazine residues, especially those of atrazine, can be found, in milk, butter and sugar). Phenylureas are replacing triazines as they are easily degraded, but this fact makes their analysis more difficult. Phenoxyacids are the oldest synthetic herbicides. Other herbicides include triazones, carbamates, uracils, pyridazines, substituted ureas and anilines (**Figure 1**).

Methods used to analyse herbicides and their residua are similar to those used for other pesticides. The methods applied should be able to determine multicomponent pesticide mixtures simultaneously, have a good reproducibility and a high recovery and a low limit of determination (the maximum permissible concentration in drinking water is as low as 0.1 μg L^{-1}).

Principles of Thin Layer Chromatography and High Performance TLC (HPTLC)

Thin-layer chromatography (TLC) remains an important practical analytical method for the analysis of herbicides with well-developed standard procedures. Its main advantages for this type of analysis are simple equipment, the possibility of varying a large number of the experimental parameters, high throughput (up to 36 samples can be analysed simultaneously), fast analyses, economy and low solvent consumption. Sample clean-up is either simple or not required at all.

TLC thus provides a simple and inexpensive screening method for the analysis of herbicides.

In classical TLC the samples are applied to the thin layer and then the layer is developed by a mobile phase (a solvent or a solvent mixture). After the mobile phase is evaporated, the separated zones are evaluated. As capillary forces govern the migration of the solute through the stationary phase, the mobile-phase velocity is less than optimal. Higher velocity can be obtained by forced-flow development.

High performance TLC (HPTLC), which has developed from classical TLC, offers greater separation efficiency, greater sensitivity and reproducibility, accurate quantification and automation.

Modern instrumental HPTLC is thus a complementary technique to high performance liquid chromatography (HPLC) in the analysis of herbicides and is increasingly used in this application.

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Chromatographic Systems

Silica gel is the most common stationary phase in TLC and HPTLC of herbicides but reversed-phases (silica gel modified with C_8, C_{18}, e.g., RP-18 W, Nano-Sil C_{18}-100, silica gel impregnated with paraffin oil) can also be used. Silica gel impregnated with diethylene glycol is suitable for triazine herbicides. Good separation of herbicides can also be obtained on alumina. TLC layers covered with a transparent polymer film have been recommended to prevent evaporation of the mobile phase and volatile herbicide samples and to suppress the adsorption of environmental impurities.

Mobile phases frequently used in the TLC of herbicides in combination with silica gel include dichloromethane, hexane–acetone and hexane–butyl acetate mixtures. Methanol or acetonitrile with water is recommended when reversed-phase stationary phases are employed. Hexane–dioxane mixtures are used with alumina layers. The hydrophobicity of herbicides can be modified by the addition of cyclodextrins.

For the TLC systems commonly used in the analysis of herbicides, see **Table 1**.

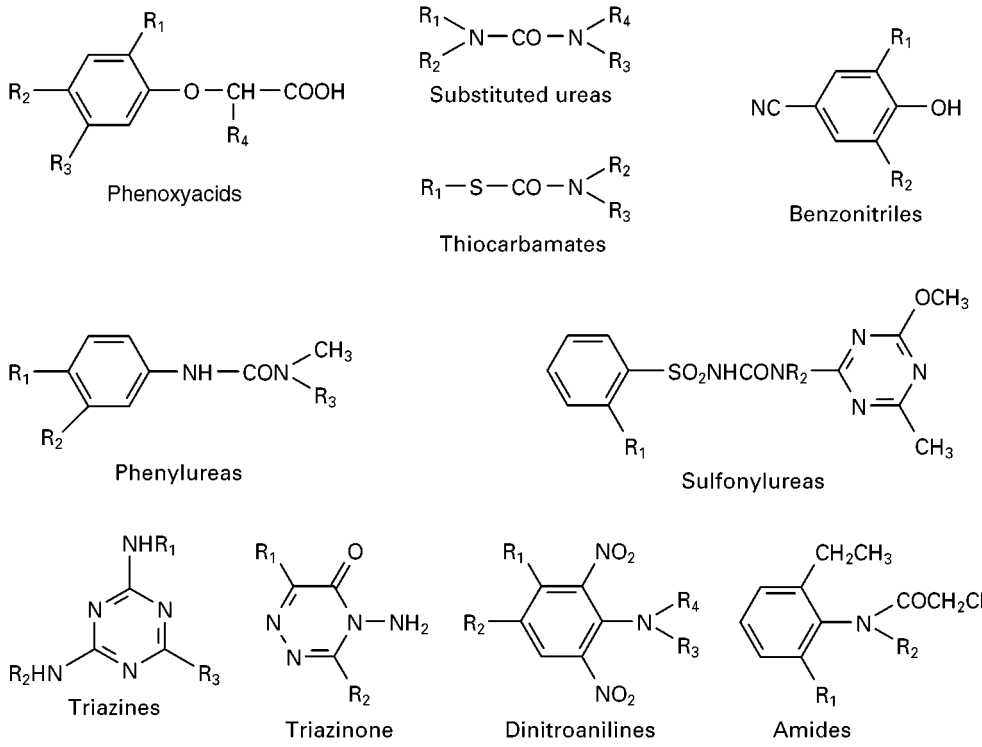

Figure 1 Structure of common herbicides.

Multiple Development

In multiple development the sample spots are re-concentrated whenever the solvent front contacts the chromatographic zone and the spots are re-focused to narrow bands. This results in increased separation efficiency and improved detection limits.

Automated multiple development (AMD) HPTLC has been applied to the screening of pesticides (including triazine, phenylurea, carbamate, phenoxycarboxylic acids and other herbicides) in environmental samples. Herbicides from different classes can be resolved by a universal gradient, based on dichloromethane. Positive results are confirmed by a second analysis using special gradients optimized for individual classes. Separated pesticides can be evaluated by densitometric detection and characterized by their UV spectra and migration distance. Most pesticides have detection limits in the range from 5 to 60 ng and their analysis does not require preconcentration methods. Only a few pesticides have higher detection limits and require preconcentration, e.g. by solid-phase extraction. An AMD-HPTLC method, DIN 38407, part 11, has been included in Germany's official methods for water analysis. An example of the AMD-HPTLC analysis of drinking water spiked with herbicides is shown in **Figure 2**; multi-wavelength detection was used. The optimized 20-step gradient for this separation is shown in **Figure 3**. Analysis takes 90 min (12 samples are analysed on one plate)

Table 1 Examples of TLC systems for the analysis of herbicides

Stationary phase	*Mobile phase*	*Detection*
Silica gel (60 or G)	Dichloromethane Ethyl acetate Hexane–acetone (8 : 2) Toluene–acetone (85 : 15) Benzene–chloroform–methanol (9 : 3 : 2) Chloroform–methanol (3 : 1)	Hill's reaction, sprayed with silver nitrate, *o*-toluidine 4,4′-tetramethyldiaminodiphenylmethane, 1% ferric chloride in butanol
PR-18W, PR-18, Nano-Sil C_{18}-100	Methanol–water (7 : 3) Acetonitrile–water (7 : 3)	Sprayed with silver nitrate or *o*-toluidine

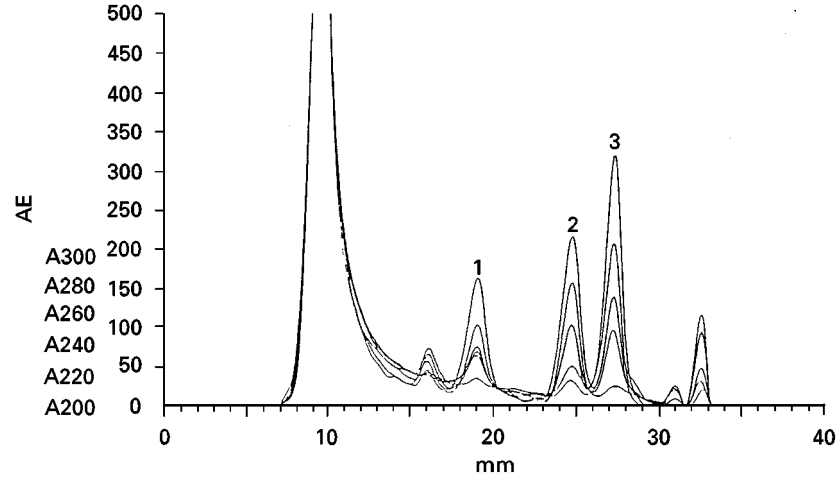

Figure 2 Multi-wavelength scan of drinking water spiked with 1, 2,4-dichlorophenoxyacetic acid; 2, mecoprop; 3, simazine. The optimized gradient is given in Figure 3. Reproduced with permission from Morlock (1996).

and peak capacity (separation number) is more than 40. This method was shown to be suitable for 283 pesticides, including herbicides.

Detection

Various agents have been used to visualize herbicide spots. They include silver nitrate, Gibbs reagent, diphenylamine and ninhydrin. The commonest detection method in TLC of herbicides is densitometry. Pre- and post-column derivatization can be carried out if the substances do not absorb visible or UV light. Simultaneous use of densitometry and fluorescence quenching is advantageous in the analysis of herbicides in multicomponent formulation, e.g. bromacil and diuron, chlotoluron and terbutryn.

Biochemical detection based on Hill's reaction is a very sensitive and selective method for herbicide residues that inhibit the enzyme system of isolated chloroplast. The concentration of the herbicide residues can also be determined from the lifetime of the spot. This method can be used for triazines, phenylureas, carbamates, uracils and pyridazone. Detection limits for herbicide residues in water, agriculture crops, foods and soil are in the range 1–10 $\mu g\ kg^{-1}$.

TLC with radioactive detection is useful in environmental studies, for example, in studies on the degradation of herbicides, formation of residues and their uptake and metabolism by plants.

Combination of TLC with mass spectrometry, recommended for the identification of herbicide metabolites, is less frequently used because of its high price.

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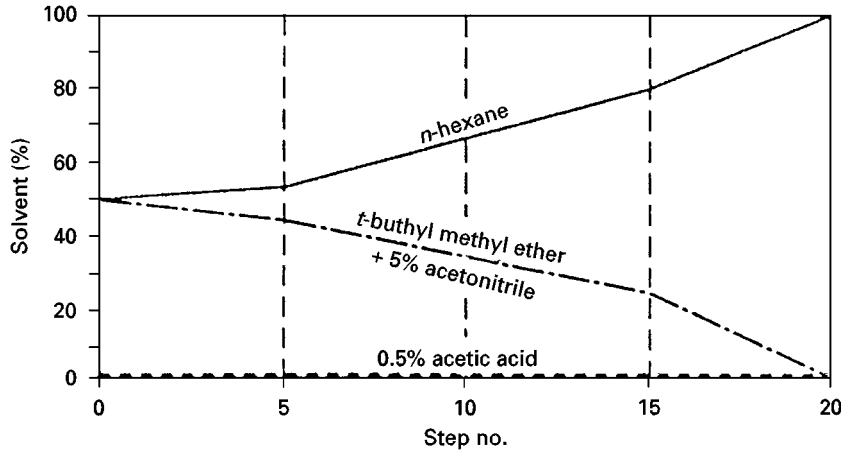

Figure 3 Optimized 20-step gradient for the determination of 2,4-dichlorophenoxyacetic acid, 4-chloro-2-methylphenoxyacetic acid (isooctyl ester) and atrazine. Reproduced with permission from Morlock (1996).

Selected Applications

TLC analysis of herbicides present in various matrices is usually preceded by isolation and/or preconcentration methods, e.g. solid-phase extraction (SPE) for water samples and supercritical fluid extraction (SFE) for solid samples, e.g. soils and plants; sample clean-up is usually not required. Some typical examples of the application of TLC in herbicide analysis are given below.

TLC can be used to determine triazine herbicides and their metabolites in drinking and surface water. Preconcentration by SPE is followed by chromatography on C_{18} plates with hexane–ethyl acetate–acetone (4 : 4 : 1 v/v) or silica gel with nitromethane–tetrachloromethane (1 : 1 v/v) and densitometric detection. The same chromatographic system can be used to determine triazine and triazone herbicides in soil after SFE. Detection limits range from 30 to 60 ng L^{-1}. A simple TLC method to determine atrazine in drinking and ground water is based on its extraction with chloroform, followed by separation on silica gel with toluene–acetone (85 : 15) as mobile phase and detection by spraying with 4,4′-tetramethyldiaminodiphenylmethane. The detection limit is 20 ng L^{-1}.

Phenoxyacetic acid herbicides can be analysed on silica gel with acidified mobile phases, e.g. ethyl acetate–acetic acid (49 : 1 v/v) or toluene–acetone–acetic acid (2 : 2 : 1 v/v) and detected by spraying with a solution of 3,5-dichloro-*p*-benzoquinonechlorimine.

Residue analysis of phenylurea herbicides in water, potatoes and soil is based on extraction with acetone or dichloromethane, clean-up on silica gel, hydrolysis to anilines and derivatization to fluorescent dansyl derivatives *in situ* on plate. This is followed by separation on silica gel with dichloromethane–methanol (99 : 1 v/v) as mobile phase and fluorescence detection. Detection limits of *ca.* 1, 20 and 200 mg kg^{-1} can be attained for water, potato and soil samples.

Pre-coated silica gel plates impregnated either with a 20% solution of formamide (A) or diethylene glycol (B), with hexane–chloroform–diethyl ether (2 : 1 : 1 v/v) as a mobile phase for system A and hexane–benzene–acetone (1 : 1 : 1) for system B gave satisfactory separation of 12 substituted urea herbicides (**Table 2**).

The screening of drinking water (about 300 compounds, including herbicides) is based on SPE followed by AMD. Identification is by analysing the sample under different separation conditions, together with reference compounds, measurement of the UV spectra *in situ* and by the use of post-chromatographic detection with various reagents. Both SPE and HPTLC can be automated.

Table 2 R_F values of substituted urea herbicides

Herbicide	*A*	*B*
N′-(3-chloro-4-methoxyphenyl)-*N,N*-dimethylurea	0.16	0.36
N′-Phenyl-*N,N*-dimethylurea	0.20	0.40
N′-(4-Chlorophenyl)-*N,N*-dimethylurea	0.26	0.47
N′-(3,4-Dichlorophenyl)-*N,N*-dimethylurea	0.35	0.54
N′-(3-Chloro-4-methylphenyl)-*N,N*-dimethylurea	0.39	0.58
N′-[4-(4′-Chlorophenoxyphenyl)]-*N,N*-dimethylurea	0.45	0.62
N′-(4-Isopropylphenyl)-*N,N*-dimethylurea	0.46	0.64
N′-(4-Chlorophenyl)-*N,N*-methoxyphenylurea	0.70	0.77
N′-(4-Bromohenyl)-*N,N*-methoxyphenylurea	0.74	0.80
N′-(3,4-Dichlorophenyl)-*N,N*-methoxy-phenylurea	0.78	0.83
N′-(4-Chlorophenyl)-*N,N*-methylisobutyl-2-urea	0.56	0.70
N′-(3,4-Dichlorophenyl)-*N,N*-methylbutylurea	0.72	0.79

Reproduced with permission from Ogierman L and Brysz G (1981) Partition thin-layer chromatography of some phenylurea herbicides. *Fresenius Zeitschrift für Analytische Chemie* 308: 464.

HPTLC can be used for the optimization of HPLC conditions where direct method development is more time-consuming and expensive. The herbicide migration data obtained in HPTLC on silica gel, RP-18, RP-8 and CN chemically bonded phases correlate well with HPLC.

Conclusions and Future Trends

TLC is useful in herbicide analysis when many samples have to be analysed. It requires minimal sample pretreatment and reduces the number of separation steps. The reason for using HPTLC for herbicide residue is that it can analyse samples in complex matrices with minimal matrix modification. The ability to separate a number of samples simultaneously is important in screening studies. HPLC should be applied in cases of complicated samples when TLC fails and when full automation is required.

It is expected that TLC will keep its place as a simple and rapid method for qualitative and semiquantitative analysis of herbicides. HPTLC will find increasing use in herbicide residue determinations due to its main advantages of increased separation efficiency, decreased detection limit, more accurate quantitative analysis and automation. There will be developments in mobile-phase optimization. Greater use of combined TLC-spectroscopic methods can be expected.

A growing demand for method validation can be expected.

See also: **II/Chromatography: Thin-Layer (Planar):** Densitometry and Image Analysis; Layers; Modes of Development: Conventional; Modes of Development: Forced Flow, Over Pressured; Spray Reagents. **Extraction:** Analytical Extractions; Solid-Phase Extraction; **III/Herbicides:** Gas Chromatography; Solid-Phase Extraction. **Impregnation Techniques: Thin-Layer (Planar) Chromatography. Pesticides:** Gas Chromatography; Thin-Layer (Planar) Chromatography.

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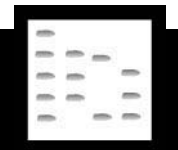
HEROIN: LIQUID CHROMATOGRAPHY AND CAPILLARY ELECTROPHORESIS



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Introduction

The separation and quantitative determination of opiates is required for a wide variety of purposes and applications. These include therapeutic drug monitoring, metabolism and pharmacokinetic studies and forensic investigations, as well as the detection and control of drug abuse. The determination of opiates in human urine is of considerable analytical interest, particularly in the context of detecting the consumption of heroin; it is in this context that the present article is written.

The first step in the establishment of such presumptive consumption of heroin is usually by enzyme immunoassay. This has the merit of low detection limit and large sample throughput, making it very suitable for large screening programmes. The immunoassay technique, however, is nonselective with respect to individual opiates and a positive result in such a screen for legal purposes must be followed by identification of individual opiates present. The purpose of this is usually to confirm or refute the hypothesis that heroin has been consumed. The short metabolic half-life of heroin complicates its confirmation, so that its consumption is inferred by detection of its metabolites. One generally sought metabolite is morphine owing to its relatively long half-life. This approach has the disadvantage that morphine is also produced as a metabolite of codeine, so that heroin consumption is presumed or not on the basis of notional codeine-to-morphine ratios. The detection of the first metabolite of morphine, 6-monoacetylmorphine, is now taken as an unequivocal indicator of heroin consumption.

The detection of heroin consumption is further complicated by the quite general inclusion of legal opiates such as codeine, pholcodine and dihydrocodeine in commonly available medicines. This results in numbers of subjects being screened as positive for opiate consumption who are not confirmed by alternative methods as having consumed heroin or morphine.

To confirm the presence of individual opiates, techniques are required that are more selective than immunoassay. These are usually based on established chromatographic techniques. Several thin-layer systems have been used but in general these have